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DOI: https://doi.org/10.1111/j.1574-6968.2012.02579.x

MINIREVIEW

Porphyromonas gingivalis: an invasive and evasive opportunistic oral pathogen

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Received 25 January 2012; revised 18 April 2012; accepted 20 April 2012.
Final version published online 28 May 2012.

DOI: 10.1111/j.1574-6968.2012.02579.x

Editor: Michael Mourez

Keywords
Porphyromonas gingivalis; inflammation; immune response; periodontitis.

Abstract

Porphyromonas gingivalis is a Gram-negative oral anaerobe that is involved in the pathogenesis of periodontitis, an inflammatory disease that destroys the tissues supporting the tooth, eventually leading to tooth loss. *Porphyromonas gingivalis* has can locally invade periodontal tissues and evade the host defence mechanisms. In doing so, it utilizes a panel of virulence factors that cause deregulation of the innate immune and inflammatory responses. The present review discusses the invasive and evasive strategies of *P. gingivalis* and the role of its major virulence factors in these, namely lipopolysaccharide, capsule, gingipains and fimbriae. Moreover, the role of *P. gingivalis* as a 'keystone' biofilm species in orchestrating a host response, is highlighted.

Introduction

Porphyromonas gingivalis and association with periodontal disease

Periodontal disease, or periodontitis, is defined as a bacterially induced inflammatory disease of the tooth-supporting (periodontal) tissues. Although more than 700 bacterial species can colonize the oral cavity (Aas *et al.*, 2005), only a handful of those are highly implicated in the disease (Paster *et al.*, 2006). *Porphyromonas gingivalis* is the species most highly associated with the chronic form of periodontitis, and can be detected in up to 85% of the disease sites (Yang *et al.*, 2004). It is detected rarely or at low in numbers in healthy sites. The presence of *P. gingivalis* in a periodontal pocket may predict imminent disease progression (van Winkelhoff *et al.*, 2002) and a significant positive correlation is found between *P. gingivalis* numbers and pocket depth (Kawada *et al.*, 2004). Following periodontal treatment, a reduction of *P. gingivalis* numbers is associated with resolution of disease at the affected site (Haffajee *et al.*, 1997; Fujise *et al.*, 2002). Moreover, experimental implantation of *P. gingivalis* in animal models induces an inflammatory response and periodontal bone loss (Evans *et al.*, 1992; Hajishengallis *et al.*, 2011). This species possesses a number of potential virulence factors, such as cysteine proteinases (gingipains), lipopolysaccharide (LPS), capsule and fimbriae (Lamont & Jenkinson, 1998). Collectively, due to these properties *P. gingivalis* is considered an 'opportunistic pathogen', in line with the modified Koch's postulates for oral infections, such as periodontal diseases (Socransky, 1979).

Structural and growth characteristics of *P. gingivalis*

Porphyromonas gingivalis is a black-pigmented, assaccharolytic, non-motile Gram-negative species that requires anaerobic conditions for growth, and the presence of heme or hemin and vitamin K in its nutrient milieu. It gains its metabolic energy by fermenting amino acids, a

property decisive for its survival in deep periodontal pockets, where sugars are extremely scarce. When considering its location in multispecies subgingival biofilm communities, *P. gingivalis* is a late colonizer, and hence is found in close proximity to and interacts with the juxtaposing gingival tissue (Kolenbrander *et al.*, 2011; Zijnge *et al.*, 2011). The black pigmentation of *P. gingivalis* colonies observed in blood agar culture is itself associated with the aggregation of heme on its cell surface (Liu *et al.*, 2004; Smalley *et al.*, 2006). This property is somehow connected to its capacity to act as an opportunistic pathogen, as when grown in a heme-limited medium it becomes less virulent (McKee *et al.*, 1986).

Invasion of the host by *P. gingivalis*

As part of its strategies for survival into the host, *P. gingivalis* is able to invade cells and tissues (Yilmaz, 2008), thus avoiding the immune surveillance. *Porphyromonas gingivalis* can actively invade gingival epithelial cells, where it can maintain viability and replicate (Belton *et al.*, 1999; Tribble *et al.*, 2006). This invasive property is dependent on its major fimbriae, which bind to β1 integrin on the surface of host cells, an event that causes rearrangements of the actin cytoskeleton to allow internalization (Yilmaz *et al.*, 2002, 2003). *Porphyromonas gingivalis* can also invade macrophages, but within these cells its replication is less active (Wang *et al.*, 2007). This is potentially a strategy for limited exposure to the extracellular environment and evasion of the immune surveillance. Interestingly, once *P. gingivalis* has invaded intracellularly, there are no signs of apoptosis or necrosis (Nakhjiri *et al.*, 2001). It can then actively secrete an ATP-hydrolysing enzyme, thus suppressing ATP-dependent apoptosis (Yilmaz *et al.*, 2008) and allowing its survival in host cells. Subsequently, it can disseminate from cell to cell, through actin cytoskeleton bridges without causing cell death, and spread while avoiding immune surveillance (Yilmaz *et al.*, 2006). Once *P. gingivalis* is established in the cell, it affects cell-cycle pathways and thus accelerates proliferation of gingival epithelial cells, in a fimbriae-dependent fashion (Kuboniwa *et al.*, 2008). This could well constitute a mechanism of expansion of the periodontal pocket epithelium, which is a histopathological feature of periodontitis.

Survival strategies of *P. gingivalis*

It is now well established that *P. gingivalis* is not an aggressor of the inflammatory response, but rather an opportunist that can cross-talk with the host and subvert its defence mechanisms. Using this strategy, *P. gingivalis* prolongs its survival and becomes established in the periodontal pocket (Hajishengallis *et al.*, 2011). It preferentially deregulates innate immunity, which may in turn disable adaptive immunity (Hajishengallis, 2009; Pathirana *et al.*, 2010). Important representative examples of these abilities are its capacity to degrade human defensins (Carlisle *et al.*, 2009), its resistance to oxidative burst-killing by polymorphonuclear neutrophils (PMNs) (Mydel *et al.*, 2006) and its ability to inhibit 'at will' the production of crucial proinflammatory cytokines (Bostanci *et al.*, 2007a, b). Although *P. gingivalis* has the capacity to stimulate interleukin (IL)-8 production by epithelial cells (Sandros *et al.*, 2000; Asai *et al.*, 2001; Kusumoto *et al.*, 2004), it can also inhibit IL-8 production, resulting in hindered PMN chemotaxis, a phenomenon known as 'chemokine paralysis' (Darveau *et al.*, 1998). *Porphyromonas gingivalis* thereby incapacitates the first line of defence in the periodontal tissues. Moreover, by inhibiting IL-12 production by macrophages, it prevents cytotoxic T-cell activation and therefore bacterial clearance (Hajishengallis *et al.*, 2007). Accordingly, by inhibiting interferon (IFN)-γ production by T cells, it inhibits macrophage bacteriocidal activity and hence bacterial clearance (Pulendran *et al.*, 2001; Hajishengallis *et al.*, 2007). A special relationship is also revealed between *P. gingivalis* and the complement system, as it can suppress its activation, that is by degradation of C3 and capturing of C4b-binging protein, but also by synergizing with C5a via exploiting toll-like receptor (TLR)-2 signalling (Wang *et al.*, 2010). A further interesting point is that whole viable *P. gingivalis* is differentially sensed by the host, compared with its released virulence factors, with the potential to activate distinctive intracellular pathways (Pathirana *et al.*, 2010), or differential cytokine production (Zhou *et al.*, 2005).

Virulence factors of *P. gingivalis*

As an opportunistic pathogen, it is not surprising that *P. gingivalis* possesses a number of virulence factors. These are molecules that can elicit deleterious effects on host cells, essentially the survival 'weapons' of *P. gingivalis*. The main virulence factors discussed here are LPS, capsular polysaccharide (CPS), fimbriae and gingipains.

The LPS of *P. gingivalis*

Like all Gram-negative bacterial species, *P. gingivalis* is sheathed by an LPS, which is an outer membrane component recognized by the host that can trigger intracellular signalling events. The affinity of LPS to its pattern recognition receptors, such as the TLRs and CD14, enables discrimination between commensal and pathogenic species. The *P. gingivalis* LPS is a stimulator of proinflammatory

responses and bone resorption, as demonstrated in experimental animal models (Chiang *et al.*, 1999; Nishida *et al.*, 2001). *In vitro*, it stimulates proinflammatory cytokine production of, for example, IL-1α, IL-1β, IL-6, IL-8, IL-18 and tumour necrosis factor (TNF)-α in monocytes (Zhou *et al.*, 2005; Bostanci *et al.*, 2007a, b; Hamedi *et al.*, 2009). Yet, *P. gingvalis* LPS exhibits controversial features with regard to the induction of an inflammatory response. Apart from being a weaker cytokine stimulator compared with the LPS of other Gram-negative (i.e. enteropathogenic) species (Liu *et al.*, 2008), it can also antagonize the cytokine-stimulating capacity of other putative pathogens (Bostanci *et al.*, 2007a, b).

Structurally, *P. gingivalis* LPS exhibits unique features compared with the LPS of other species. These include differences in the structure of the O-antigen between *P. gingivalis* strains that can confer antigenic differences (Paramonov *et al.*, 2001, 2009), as well as in the acylation patterns and receptor-activating capacities of the lipid A component. While the lipid A of most Gram-negative species is a strong activator of TLR4 responses, *P. gingivalis* lipid A is predominantly a TLR2 activator and may even act as antagonist to TLR4 (Darveau *et al.*, 2004), dampening the immune responses (Hajishengallis, 2009). When considering further the heterogeneous acylation patterns of *P. gingivalis* lipid A, two forms are predominant: the tetra-acylated and penta-acylated forms. These two structures induce opposing host responses. The penta-acylated lipid A activates TLR4, whereas tetra-acylated lipid A acts as a TLR4 antagonist (Darveau *et al.*, 2004; Nemoto *et al.*, 2006). These changes of *P. gingivalis* lipid A acylation are dependent on microenvironmental conditions. In particular, when hemin availability is high (a condition that reflects inflammation), penta-acylated lipid A is converted into tetra-acylated lipid A (Al-Qutub *et al.*, 2006). Hence, by modifying its lipid A structure according to the microenvironment, *P. gingivalis* may modulate the binding affinity of its LPS to its cognate TLR receptors, subsequently selecting how to affect downstream host immune signalling. Interestingly, a second type of LPS has also been identified in *P. gingivalis*, containing a distinct anionic polysaccharide linked to lipid A, known as A-LPS (Paramonov *et al.*, 2005). A-LPS is required for cell integrity and serum resistance (Shoji *et al.*, 2002; Paramonov *et al.*, 2005; Slaney *et al.*, 2006) and is structurally associated with the Arg-X gingipain (Curtis *et al.*, 1999; Paramonov *et al.*, 2005). It is also a weaker inducer of cytokine responses by human monocytes, as compared with the conventional LPS (Rangarajan *et al.*, 2008). Collectively, the modifications and heterogeneity of *P. gingivalis* LPS can result in opposing actions and immunological deregulation. Strategically, this is in line with the manipulation of host innate immune responses by this species, to facilitate its adaptation and survival into the host.

The CPS of *P. gingivalis*

A major virulence factor of *P. gingivalis* is considered to be its capsule, also known as CPS or K-antigen (Schifferle *et al.*, 1989; Holt *et al.*, 1999; Farquharson *et al.*, 2000; Aduse-Opoku *et al.*, 2006; Brunner *et al.*, 2010a, b). Based on the capacity of CPS to generate systemic IgG antibody responses, at least six different serotypes have been identified (Laine *et al.*, 1997; Sims *et al.*, 2001). Encapsulated *P. gingivalis* strains are shown to be highly invasive, causing spreading infection in a murine lesion model, whereas nonencapsulated strains induced only localized abscesses (Laine & van Winkelhoff, 1998). Interestingly, immunization with *P. gingivalis* CPS induced a high IgG systemic response (Choi *et al.*, 1998) and reduced *P. gingivalis*-induced alveolar bone loss (Gonzalez *et al.*, 2003). Encapsulated strains of *P. gingivalis* are more resistant to phagocytosis by polymorphonuclear leukocytes than nonencapsulated strains (Sundqvist *et al.*, 1991) and have differential capacities to adhere to gingival epithelial cells (Dierickx *et al.*, 2003). Moreover, differences in CPS serotypes can reflect differential capacities in chemokine stimulation by macrophages (d'Empaire *et al.*, 2006) or cytokine stimulation by dendritic cells (Vernal *et al.*, 2009). Interestingly, a nonencapsulated *P. gingivalis* knockout mutant strain was found to be a more potent inducer of cytokine synthesis by human gingival fibroblasts, as compared with the corresponding wild-type strain, implying a role of CPS in downplaying the innate immune responses (Brunner *et al.*, 2010a, b). Although it is evident that the presence of CPS, or its individual serotypes, could be determinants of the virulence of *P. gingivalis*, the potential involvement of this antigen in the overall deregulation of host responses awaits further clarification.

The fimbriae of *P. gingivalis*

The fimbriae of *P. gingivalis* are thin, filamentous cell-surface protrusions that facilitate its adherence to salivary proteins, extracellular matrix, eukaryotic cells and bacteria of either the same or other species. Through its fimbriae, *P. gingivalis* can thus attach to early colonizing bacteria, and participate in the developing biofilm structure. Type I (major) fimbriae have important roles in colonization and invasion, whereas type II (minor) fimbriae possess a higher proinflammatory capacity (Lamont & Jenkinson, 1998; Amano *et al.*, 2004; Hajishengallis *et al.*, 2008). Interestingly, however, *P. gingivalis* strains W50 and W83 that lack major fimbriae are still invasive, as

demonstrated in experimental subcutaneous abscess models (Inaba *et al.*, 2008). A particular role of fimbriae is revealed in the induction of bone destruction in experimental periodontitis models. To this extent, infection of rats with nonfimbriated *P. gingivalis* strains exhibited reduced periodontal bone loss, compared with infection with fimbriated strains (Jotwani & Cutler, 2004). Moreover, immunization against *P. gingivalis* fimbriae protected against bone loss in gnothobiotic rats (Malek *et al.*, 1994; Sharma *et al.*, 2001). Other properties of both major and minor fimbriae are the induction of proinflammatory cytokines and production of matrix metalloproteinases (MMPs), such as IL-1, IL-6, IL-8, TNF-α and MMP-9, by various host cells (Jotwani *et al.*, 2010; Ogawa *et al.*, 1994; Pollreisz *et al.*, 2010; Takahashi *et al.*, 2006).

Porphyromonas gingivalis fimbriae can signal through either TLR2 or TLR4. Activation of TLR2 by fimbriae results in a differential signalling pattern compared with activation by *P. gingivalis* LPS (Hajishengallis *et al.*, 2006). Fimbriae can directly induce two distinct signalling pathways, one that mediates production of proinflammatory cytokines, such as IL-6 and TNF-α, and another that mediates the expression of cell adhesion molecules, such as ICAM-1 (Hajishengallis *et al.*, 2009). On the other hand, signalling through TLR4 requires an additional costimulation of CD14 and MD-2 (Davey *et al.*, 2008). Interestingly, major fimbriae can exploit TLR2 signalling in order to interact with complement receptor 3 (CR3), in a novel 'inside-out' signalling pattern (Hajishengallis *et al.*, 2007; Wang *et al.*, 2007). This interaction activates the binding capacity of CR3 and allows for internalization of *P. gingivalis* in macrophages and reduction of IL-12 production, which may collectively inhibit bacterial clearance (Hajishengallis *et al.*, 2007).

The gingipains of *P. gingivalis*

Gingipains are a group of cell surface cysteine proteinases of *P. gingivalis* that can also be present in secreted soluble form. They account for 85% of the total proteolytic activity of *P. gingivalis* (Potempa *et al.*, 1997). Based on their substrate specificity, they are divided into arginine-specific (Arg-X) and lysine-specific (Lys-X) gingipains (Curtis *et al.*, 2001; Guo *et al.*, 2010). Arg-X gingipains have trypsin-like activity, and can degrade extracellular matrix components, including the integrin–fibronectin-binding, cytokine, immunoglobulin and complement factors. There are two types of Arg-X gingipains, namely RgpA, which contains a proteolytic and an adhesion domain, and RgpB, which contains only the proteolytic domain. There is one type of Lys-X gingipain, Kgp, which contains both a proteolytic and an adhesion domain. There are sequence similarities between the adhesion domains of Kgp and RgpA (Curtis *et al.*, 2001).

The gingipains have multiple effects on the molecular components of the immune response, and as such they can deregulate these responses. For instance, they can cleave several T-cell receptors, such as CD2, CD4 and CD8 (Kitamura *et al.*, 2002), thereby hampering the cell-mediated immune response. They can also stimulate expression of protease-activated receptors in neutrophils (Lourbakos *et al.*, 1998), gingival epithelial cells (Lourbakos *et al.*, 2001), gingival fibroblasts and T cells (Belibasakis *et al.*, 2010), which are crucial for the induction of cytokine responses and the establishment of chronic inflammation in periodontitis (Holzhausen *et al.*, 2010; Fagundes *et al.*, 2011). Gingipains can also stimulate IL-6 production by oral epithelial cells (Lourbakos *et al.*, 2001) and IL-8 production by gingival fibroblasts (Oido-Mori *et al.*, 2001), enhancing the inflammatory responses. However, they can also proteolytically inactivate both anti-inflammatory (IL-4, IL-5) and pro-inflammatory (IL-12, IFN-γ) cytokines (Yun *et al.*, 1999, 2001, 2002; Tam *et al.*, 2009).

A number of particularly interesting effects are exerted by the gingipains on components of the complement system. Arg-X gingipains can cleave the C5 molecule, resulting in release of its C5a component, which is crucial for enhancing the recruitment of PMNs (Wingrove *et al.*, 1992; Imamura *et al.*, 2001). On the other hand, Lys-X can inactivate the C5a receptor on PMNs, an action that may actually impair their recruitment (Jagels *et al.*, 1996a, b). Along this line, the Arg-X gingipains can degrade the C3 molecule, potentially contributing to decreased bacterial opsonization (Schenkein *et al.*, 1995). This property could confer increased resistance of *P. gingivalis* to bactericidal activity.

Apart from their effect on immune responses, gingipains may also be involved in the binding of *P. gingivalis* to host cells, as Rgp–Kgp complexes have been shown to mediate adherence on gingival epithelial cells and gingival fibroblasts (Chen *et al.*, 2001; Grenier *et al.*, 2003; Andrian *et al.*, 2004). Interestingly, when *P. gingivalis* intracellularly invades gingival epithelial cells, expression of gingipain is downregulated (Xia *et al.*, 2007).

Gingipains may also affect vascular permeability and bleeding at the periodontal site. They can proteolytically activate plasma kallikrein and bradykinin, or alternatively increase the release of thrombin and prothrombin, which can result in increased vascular permeability and PMN influx (Imamura *et al.*, 1994, 1995a). Moreover, by degrading fibrinogen (Scott *et al.*, 1993), they may contribute to inhibition of blood coagulation and increase bleeding at the site (Imamura *et al.*, 1995b) , thus enhancing the availability of hemin required for *P. gingivalis* growth.

Collectively, studies in various experimental systems indicate that gingipains have seemingly contradicting

actions on the innate immune responses, hampering interpretation of their role in the pathogenesis of periodontitis. Nevertheless, such differences may be reconciled by the existence of a concentration gradient of gingipains in the tissue (Pathirana *et al.*, 2010). Closer to the gingival epithelial barrier where the biofilm resides, gingipain concentrations are high, causing degradation or deregulation of various components of the immune response. This hampers bacterial clearance and facilitates bacterial invasion. Nevertheless, deeper into the gingival connective tissue, gingipain concentrations become gradually lower and stimulate, rather than inhibit, inflammation. This may in turn induce connective tissue and bone destruction, which are the hallmarks of periodontitis.

Conclusions

It is evident that *P. gingivalis* has developed mechanisms to invade and persist into the host, by astutely adapting to its local niche. Its paradoxically opposing (stimulatory vs. inhibiting) effects on innate immune and inflammatory responses aim to subvert host defence mechanisms, in order to facilitate its survival in the tissues (Hajishengallis, 2009; Hajishengallis & Lambris, 2011). The net effect of this deregulated equilibrium is likely to determine if site-specific disease progresses beyond or remains at stationary phase. Whether inflammation is beneficial for *P. gingivalis* may depend on the stage of its establishment in the host (Hajishengallis, 2009; Pathirana *et al.*, 2010). At early stages, suppression of inflammation and evasion of host recognition would aid *P. gingivalis* in colonizing, invading and establishing at the targeted site. At later stages, once *P. gingivalis* is well established, inducing inflammation may facilitate its increased demands in nutrients. Alternatively, *P. gingivalis* may induce a 'nonproductive inflammation', one that fails to eliminate it, yet is sufficient to induce mediators of tissue destruction (Hajishengallis, 2009).

Finally, as periodontitis is of polymicrobial nature, it is reasonable to consider the role of different bacterial species within the context of (subgingival) biofilm communities. Hence, *P. gingivalis* is likely to function in concerted action with other species, to their mutual benefit. For instance, complement manipulation by *P. gingivalis* may denote a coevolution strategy to support other species present in the biofilm, which may reciprocally provide further colonization opportunities and nutrient availability to *P. gingivalis*. Subsequent changes in the local microenvironment can differentially regulate expression of its virulence factors, and hence the proinflammatory or anti-inflammatory potentials of *P. gingivalis*. This is strongly indicated by recent evidence demonstrating that even at low abundance, this species qualitatively and quantitatively affects the composition of the oral commensal microbiota, which are in turn required for *P. gingivalis*-induced inflammatory bone loss (Hajishengallis *et al.*, 2011). For these reasons, *P. gingivalis* is now considered a 'keystone' species in subgingival biofilms (Honda, 2011).

Acknowledgements

This work was supported by the Institute of Oral Biology, Center of Dental Medicine, University of Zürich.

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